University of Thi-Qar Journal of Science (UTJsci)
E-ISSN: 2709-0256, P-ISSN: 1991-8690, Vol. 10, No. 2, Dec. 2023

The production bioethanol from *Ceratophyllum demersum* L . in Iraq

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Received: 2023-07-06, Revised: 2023-07-21, Accepted: 2023-07-21, Published: 2023-12-22

***Abstract*— Biofuels have attracted a lot of attention due to the growing demand for energy resources and concerns about greenhouse gas emissions. Unlike other green energy resources, biofuels like bioethanol, can provide liquid fuels that is essential for transportation. Second-generation bioethanol can be produced from lignocellulosic biomass through acid hydrolysis and fermentation. Lignocellulosic biomass is widely available and does not affect on the nutritional needs of agricultural crops. In this study, the aquatic plant *Ceratophyllum demersum* was used. *Ceratophyllum demersum* is a type of invasive aquatic plant that can live in fresh and brackish waters, and it is abundant in most regions of southern Iraq. The bioethanol with the highest concentration was identified by high-performance liquid chromatography (HPLC). The results showed that 14% of bioethanol was produced in the absence of acid hydrolysis, while the concentration of it increased to 25% with the presence of acid hydrolysis. Acid hydrolysis aims to increase the breaking bonds of lignin and hemicellulose, increase the porosity of the material, and damage the crystalline structure of cellulose, and thus facilitates its conversion to glucose and increases the percentage of ethanol production**.

***Keywords*—Bio-ethanol, *Ceratophyllum demersum L.*, Biofuel, Acid hydrolysis, styling.**

I. Introduction

At the global level, scientists and researchers are increasingly interested in production of biofuels from various sources of biomass in a safe way for the environment [1]. In the 20th century, energy consumption increased dramatically due to the explosive growth in population and the development of technology, which led to excessive consumption of traditional energy sources represented by fossil fuels (coal, oil, and gas). Fossil fuel consumption represents more than 80% of total energy consumption [2]. On the other hand, excessive consumption of fossil fuels leads to an increase in greenhouse gas emissions, especially carbon dioxide (CO2) [3]. These emissions lead to climate change and much environmental damage, such as global warming, floods, hurricanes, heavy rains, and many other severe weather conditions [4].

Biofuel production technologies depend mainly on soil organic carbon sequestration (SOC), carbon capture and storage (CCS). Thus, biofuel production technologies can reduce greenhouse gas emissions more efficiently than any other technology [5-6]. Biofuel is defined "as fuel produced directly or indirectly from biomass such as fuel wood, coal, bioethanol, biodiesel, or biogas such as methane or biohydrogen" [7]. Although there are many ways to obtain clean energy from wind, water, and the sun, the use of biomass is very important for the generation of biofuels because, unlike other renewable energy sources, it produces liquid fuel for transportation [8]. Biofuels are classified into two categories: primary biofuels and secondary biofuels [9]. Primary biofuels are produced directly from untreated forest wood, agricultural crop residues, plants, and animal wastes [10]. Secondary biofuel, it is obtained from biomass after processing it using microorganisms or nanomaterials [11]. Secondary biofuels are divided into four generations, depending on the type of feedstock (biomass) and production methods [12]. Second-generation bioethanol produced from biomass such as agricultural waste, municipal waste, aquatic plants, etc. has gained a lot of attention in recent years. Lignocellulosic biomass has a great potential to be used as feedstock for bioethanol production because it does not affect on human nutritional needs, in addition to its cheapness, availability, and sustainability [13].

A. Bioethanol production from Ceratophyllum demersum biomass

Ceratophyllum demersum, shown in Figure. 1, is a submerged aquatic plant common throughout the world. It does not have roots, but it can establish itself using modified, root-like leaves [14]. It is classified as one of the most harmful invasive aquatic freshwater plants in the world

https://doi.org/10.32792/utq/utjsci/v10i2.1082

[15]. According to its high growth rate, it has a lot of potential environmental and economic damage, so converting it into biofuel has a benefit for water treatment. This type of plant contains a high percentage of carbohydrates (about 40%), so it is considered a suitable raw material for the production of alcohol [16].

Figure 1: *Ceratophyllum demersum* L.

Thus, the current study aims to take advantage of the natural resources in Iraq and produce bioethanol from Ceratophyllum demersum.

II. METHODOLOGY

A. Sample collection and initial Pretreatment

Plant samples of *Ceratophyllum demersum* were collected on September 13, 2022, from the general estuary in the city of Nasiriyah. Then, the samples were washed thoroughly with clear water repeatedly to get rid of mud and suspended materials and dried them on a white paper surface in shade area for 7 days. After it was completely dried, it was ground using an electric mill into a fine powder of pale green color and lightweight.

B. Determination of the chemical content of Ceratophyllum demersum

Physically treated plant powder was used to estimate the proportion of important compounds in the biomass. The total carbohydrate content was estimated using the Phenol-Sulfuric Acid Method [17]. The total fat in the biomass was also estimated using the method presented in [18]. Finally, the total ash content was estimated according to the method presented by [19], the moisture content according to the method presented in [20].

C. Acidolysis and fermentation

The fermentation process for the sample powder was carried out in two steps:

The first step: In this part, three different quantities (2.5g, 5 g, and 7.5g) of the sample powder were subjected to a fermentation process only. Appropriate experimental conditions were chosen based on the previous study [16], with a pH value of 6, a temperature of 30° C, and 10% (w/v) of Saccharomyces cerevisiae. This fermentation process was conducted at three different times (24 h, 48 h, and 72 h).

The second step: This step includes two procedures: the first step is hydrolysis in the presence of a hydrochloric acid catalyst (HCl). Based on a previous study [21], the acidolysis of 2g and 5g of the solid was carried out using three different concentrations of HCl acid (1M, 2M, and 3M). at different. times (48 h, 72 h) and a temperature of 45 °C. These different parameters were used to determine the best concentration of acid used in the production of optimal reducing sugars. The effectiveness of the acid hydrolysis process can be seen qualitatively by comparing the color change in the solution before and after hydrolysis. Where the color of the solution becomes bright red [21]. The second process is the fermentation of the acid decomposition product using 10% (w/v) of yeast (Saccharomyces cerevisiae) for a period of 48 h. It was observed that a hazy precipitation with a white color formed at the bottom of the conical flask.

D. Analytical methods

Ethanol content from the fermentation was determined using HPLC. The analysis condition was using Kinesis, Telos, C18, 5µm, 300×3.9mm USA column temperature was 25 degree Celsius; mobile phase was Acetonitrial 99.0%, Methmal 10% and was fed with flow rate of (1.0ml/min).Ethanol concentration was identified by comparison with standard solutions.

III. RESULTS AND DISCUSSIONS

A. The chemical composition of Ceratophyllum demersum L.

The need for renewable and clean energy resources encouraged researchers to work on alternative resources like biomass. C. demersum can be used as lignocellulosic biomass to generate liquid fuel (bioethanol) [15]. The total carbohydrate content that was produced in this study was about 44.09%, As shown in Table 1. This value indicates the high sugar content of the biomass that can be used to produce bioethanol [16].

Table 1: Chemical composition of C. demersum biomas

Chemical composition	Percentage
Total lipid	0.60
Total carbohydrate	44.09
Moisture	8.50
Ash	18.00

Because *C. demersum has a* high content of carbohydrates, it can be used to manufacture bioethanol by going through the hydrolysis step in the presence of an acidic HCl catalyst with a concentration difference of 1M, 2M, and 3M and a decomposition temperature of 48°C. Then, the lysis product was fermented with 10% (w/v) of Saccharomyces cerevisiae for 48 hours. Moreover, the production of bioethanol without acidolysis of biomass was tested with time changes of 24 hours, 48 hours, and 72 hours and a constant temperature of 30 °C.

B. Acidolysis and fermentation

The first step: the maximum yield of bioethanol was checked using our desired parameters, like the amount of solid matter and the fermentation time by using the HPLC, The fermentation of 2.5% (w/v) of the solid matter produced a maximum ethanol production during 48 hours at a rate of 14.661%; while, alcohol content decreased to 0% at 72 hours for all three quantities of solid matter (2.5, 5, and 7.5 g). It was noted from Table 2 that as the amount of solid matter (plant powder) increased, the amount of alcohol decreased over a time of 48 h, where the amount of alcohol was (14.661%) with 2.5% (w/v) of the solid, 2.31% (w/v) with 5% of the solid, and 1.522% with 7.5% (w/v) of the solid. This result was because when there is a high concentration of the solid, it inhibits yeast growth, which leads to a low concentration of alcohol [22].The results are shown in Table 2.

Table 2: The amount of bioethanol obtained from the fermentation process in the first step using the HPLC

Time (h)	Substrate quantity (w/v)	Concentrations (m.molary)%	Retention time (min)
24	2.5%	1.287	9 ± 0.4
48	2.5%	14.661	9 ± 0.4
72	2.5%	0	9 ± 0.4
24	5%	1.532	9 ± 0.4
48	5%	2.31	9 ± 0.4
72	5%	0	9 ± 0.4
24	7.5%	1.393	9 ± 0.4
48	7.5%	1.522	9 ± 0.4
72	7.5%	0.089	9 ± 0.4

The second step: Acid hydrolysis leads to increase the breaking of the bonds of lignin and hemicellulose, the porosity of the material and damage the crystalline structure of cellulose, which facilitates its conversion to glucose [23]. As in the previous batch, samples were analyzed using HPLC. 3M concentration achieved the highest ethanol yield of 23.42% when the amount of solid matter was 2.5% (w/v) as shown in Table 3. The concentrated acid process generates a high sugar recovery [24]. While the 2M concentration achieved the highest ethanol yield by 25% when the amount of solid matter was 5% (w/v). High solids amount of 5% (w/v) affected the acidolysis and fermentation processes. High loading of biomass leads to a decrease in the contact surface between the acid and the substrate, which leads to a decrease in the sugar concentration and the efficiency of acid hydrolysis [25]. A previous study also confirmed that a high concentration of ethanol causes inhibition of yeast growth and thus inhibits ethanol production [26]. The results are shown in Table 3.

Table 3: The amount of bioethanol obtained from acid hydrolysis and fermentation in the second step using HPLC

HCl concentrations (M)	substrate quantity (w/v)	Concentrations (m.molary)%	Retention time (min)
1M	2.5%	13.17	3 ± 0.3
2M	2.5%	8.00	3 ± 0.3
3M	2.5%	23.42	3 ± 0.3
1M	5%	2	3 ± 0.3
2M	5%	25	3 ± 0.3
3M	5%	0	3 ± 0.3

IV. Conclusion

The present study demonstrated that the aquatic plant C. demersum is a novel and renewable substitute for bioethanol. Bioethanol can be produced from C. demersum, which is abundant in Iraq. This type of plant contains an appropriate carbohydrate ratio, and thus can benefit from natural resources and direct them towards a sustainable energy source. This study recommends that the accumulation of aquatic wastes that cause economic damage can be effectively eliminated by processing the biomass of aquatic weeds into value-added products.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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